

Extracorporeal Carbon Dioxide Removal Enhanced by Lactic Acid Infusion in Spontaneously Breathing Conscious Sheep

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ABSTRACT

Background: The authors studied the effects on membrane lung carbon dioxide extraction (VCO_2 ML), spontaneous ventilation, and energy expenditure (EE) of an innovative extracorporeal carbon dioxide removal (ECCO₂R) technique enhanced by acidification (acid load carbon dioxide removal [ALCO₂R]) *via* lactic acid.

Methods: Six spontaneously breathing healthy ewes were connected to an extracorporeal circuit with blood flow 250 ml/min and gas flow 10 l/min. Sheep underwent two randomly ordered experimental sequences, each consisting of two 12-h alternating phases of ALCO₂R and ECCO₂R. During ALCO₂R, lactic acid (1.5 mEq/min) was infused before the membrane lung. Caloric intake was not controlled, and animals were freely fed. VCO_2 ML, natural lung carbon dioxide extraction, total carbon dioxide production, and minute ventilation were recorded. Oxygen consumption and EE were calculated.

Results: ALCO₂R enhanced VCO_2 ML by 48% relative to ECCO₂R (55.3 ± 3.1 vs. 37.2 ± 3.2 ml/min; P less than 0.001). During ALCO₂R, minute ventilation and natural lung carbon dioxide extraction were not affected (7.88 ± 2.00 vs. 7.51 ± 1.89 l/min, $P = 0.146$; 167.9 ± 41.6 vs. 159.6 ± 51.8 ml/min, $P = 0.063$), whereas total carbon dioxide production, oxygen consumption, and EE rose by 12% each (223.53 ± 42.68 vs. 196.64 ± 50.92 ml/min, 215.3 ± 96.9 vs. 189.1 ± 89.0 ml/min, 67.5 ± 24.0 vs. 60.3 ± 20.1 kcal/h; P less than 0.001).

Conclusions: ALCO₂R was effective in enhancing VCO_2 ML. However, lactic acid caused a rise in EE that made ALCO₂R no different from standard ECCO₂R with respect to ventilation. The authors suggest coupling lactic acid–enhanced ALCO₂R with active measures to control metabolism. (ANESTHESIOLOGY 2016; 124:674-82)

EXTRACORPOREAL carbon dioxide removal (ECCO₂R) is a low blood flow extracorporeal gas exchange technique used to remove carbon dioxide in patients affected by respiratory failure. It mitigates respiratory acidosis,¹ minimizes the ventilatory burden of patients at risk of ventilator-induced lung injury, and reduces the work of breathing.² Formerly, ECCO₂R was carried out with older technology and, similar to full extracorporeal membrane oxygenation, was used as a rescue therapy for severe cases of acute respiratory distress syndrome.³ Recent technological advances have reduced the complexity of ECCO₂R^{4,5} and thus allowed this technique to be also used in acute exacerbation of chronic obstructive pulmonary disease,⁶ as a bridge to lung transplant,⁷ and to facilitate lung-protective ventilation.⁸ Nonetheless, current ECCO₂R

What We Already Know about This Topic

- Extracorporeal carbon dioxide removal is used for lung protection in patients with hypercapnic respiratory failure. Current extracorporeal carbon dioxide removal technology has low efficiency and thus requires significant invasiveness to be clinically effective.

What This Article Tells Us That Is New

- In a study of six spontaneously breathing conscious sheep connected to a minimally invasive circuit, extracorporeal blood acidification with lactic acid (acid load carbon dioxide removal) increased extracorporeal carbon dioxide removal by 50% compared with standard extracorporeal carbon dioxide removal. Although lactic acid infusion increased the overall energy expenditure, feasibility safety and efficiency of acid load carbon dioxide removal were proved.

This article is featured in "This Month in Anesthesiology," page 1A. Corresponding article on page 532. Supplemental Digital Content is available for this article. Direct URL citations appear in the printed text and are available in both the HTML and PDF versions of this article. Links to the digital files are provided in the HTML text of this article on the Journal's Web site (www.anesthesiology.org).

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technology requires the use of blood flow (BF) of at least 0.5 to 1 l/min, and catheters of 16 to 19 F size, to remove a significant portion of the total carbon dioxide production of an adult patient.^{9,10} If carbon dioxide removal could be further enhanced such that reduced BF as low as 250 to 500 ml/min became possible, smaller cannulas could be used (e.g., 13 to 15 F). Accordingly, ECCO₂R could become a procedure with the same logistical footprint and invasiveness as continuous renal replacement therapy.

In pursuit of this goal, we developed a new ECCO₂R technique consisting of regional blood acidification (acid load carbon dioxide removal [ALCO₂R]),¹¹ based on infusion of lactic acid (LA) extracorporeally into the blood entering the membrane lung (ML). Acidification converts bicarbonate ions into dissolved carbon dioxide. This increases the partial pressure of carbon dioxide (P_{CO₂}) in the blood entering the ML, raises carbon dioxide availability, and increases the transmembrane carbon dioxide partial pressure gradient. Because this is the driving force for carbon dioxide transfer across the membrane,¹² acidification enhances ML carbon dioxide removal (VCO₂ML). Previously, we demonstrated regional blood acidification to be capable of raising VCO₂ML by up to 70% (i.e., to 170 ml/min), with an extracorporeal BF as low as 250 ml/min in a mechanically ventilated porcine model.^{11,13}

Until now, ALCO₂R has been performed by infusing a metabolizable acid, such as LA, citric acid, or acetic acid.^{11,14} All three compounds are energy substrates, and therefore, their oxidation produces carbon dioxide, which may counterbalance the enhanced carbon dioxide removal effect provided by ALCO₂R. In this study, we examined the effects of ALCO₂R on spontaneous ventilation and energy metabolism of LA in the absence of caloric control. We compared ventilation and energy expenditure (EE) during ALCO₂R and standard ECCO₂R, in spontaneously breathing, freely fed, healthy ewes. We hypothesized that ALCO₂R enhances carbon dioxide removal of the ML and reduces minute ventilation (MV). Moreover, we studied the safety profile of ALCO₂R, in terms of histological damage and signs of tissue inflammation and oxidative stress.

Materials and Methods

This study was approved by the U.S. Army Institute of Surgical Research Institutional Animal Care and Use Committee (Fort Sam Houston, San Antonio, Texas) and was conducted in compliance with the Animal Welfare Act, the Implementing Animal Welfare Regulations, and in accordance with the principles of the Guide for the Care and Use of Laboratory Animals.

Under general anesthesia, six healthy ewes (*Ovis aries*, David Josh Talley, Uvalde, Texas) (34.3 ± 1.5 kg) were instrumented as described in our previous article.¹⁵ Tracheostomy was performed. Catheters were placed in the carotid and pulmonary arteries for pressure monitoring and sample withdrawal. A dual-lumen catheter (15.5 F; ALung; ALung

Technologies, USA) was introduced in the right external jugular vein for connection to the extracorporeal circuit (for additional details on instrumentation, see Instrumentation, Additional Methods, Supplemental Digital Content 1, <http://links.lww.com/ALN/B237>).

A custom-made extracorporeal circuit optimized for ALCO₂R was used for the study. Figure 1 shows the schematic representation of extracorporeal circuit. The circuit consisted of a Hemolung device (ALung; ALung Technologies), a standard polyethersulfone continuous renal replacement therapy hemofilter (Purema; NxStage Medical, USA) connected in series after the Hemolung ML, and a peristaltic pump for the recirculation of ultrafiltrate before the ML. BF was maintained constant at 250 ml/min, sweep gas flow was 10 l/min of ambient air, and ultrafiltrate flow was 100 ml/min. An acid injection port was located on the ultrafiltrate side of the circuit, as such avoiding direct contact between highly concentrated acids and the cellular components of the blood. This prevented hemolysis and unwarranted infusion of free water to dilute the acid. Six sampling outlets were arranged in the circuit: four on the blood side (inlet, postrecirculation, post-ML, and outlet) and two on the ultrafiltrate side (pre-acid, post-acid). Extracorporeal BF was measured by the Hemolung built-in flowmeter at the circuit outlet.

After surgery, anesthesia was discontinued and animals were moved into a cage. For the remainder of the study, continuous infusions of fentanyl and midazolam (0.5 µg · kg⁻¹ · h⁻¹ and 0.01 mg · kg⁻¹ · h⁻¹, respectively) were provided. Maintenance fluid (Plasma-Lyte A; Baxter International, USA) was infused at 1 ml · kg⁻¹ · h⁻¹. During the experiment, animals were conscious and breathing spontaneously, connected to a mechanical ventilator (Dräger Evita XL; Dräger Medical, Germany) in continuous positive airway pressure mode at 5 cm H₂O with an FIO₂ of 21%. Hay and dry food pellets were provided *ad libitum* throughout the study.

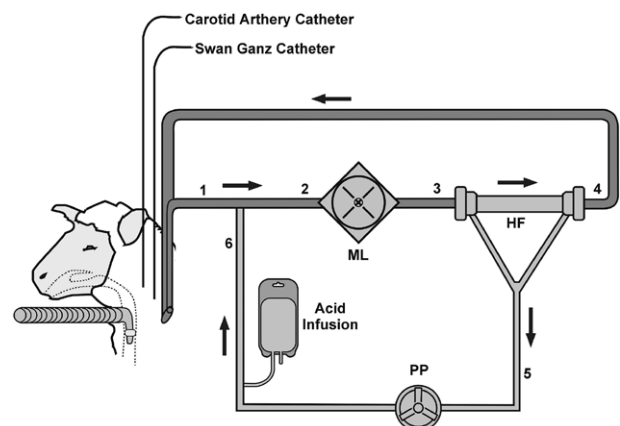


Fig. 1. Schematic representation of extracorporeal circuit. Membrane lung (ML), hemofilter (HF), and peristaltic pump (PP). 1–4 = circuit blood withdrawal sites: (1) inlet, (2) postrecirculation, (3) post-ML, and (4) outlet. 5–6 = ultrafiltrate withdrawal sites: (5) pre-acid, (6) post-acid. Flowmeter was positioned on the blood line after the outlet (4) withdrawal site.

After a recovery period of 6 h, each of the six animals was subjected to two repeated experimental sequences. Each of them lasted 24 h and consisted of a phase of ALCO₂R and a phase of standard ECCO₂R. The ALCO₂R and ECCO₂R phases were alternated, ordered randomly, and lasted 12 consecutive hours each (for additional details on experimental setup, see Experimental Design and fig. S1, Additional Methods, Supplemental Digital Content 1, <http://links.lww.com/ALN/B237>). To counterbalance any order effect of the experimental sequences, three animals started the experiment with a standard ECCO₂R phase, whereas three started with an ALCO₂R phase. We did not perform a washout period after ALCO₂R and before commencing ECCO₂R because in a previous similar study¹⁴ we observed LA (infused at 1.5 mEq/min) to be completely washed-out by sheep in less than 1 h. ALCO₂R was achieved by infusing LA (4.4 M) in the ultrafiltrate side of the circuit, at a continuous fixed rate of 1.5 mEq/min.

A volumetric capnograph (CO₂SMO; Novamatrix, USA) was used to measure respiratory rate, tidal volume (TV), MV, and natural lung carbon dioxide removal (VCO₂NL) at body temperature, ambient pressure, saturated (with water vapor) (BTPS) conditions. Alveolar ventilation (AV), physiologic dead space (V_d), and venous admixture (Qs/Qt) were calculated using standard equations (see equations A for Ventilatory Function Calculations, Supplemental Digital Content 1, <http://links.lww.com/ALN/B237>).^{16,17} VCO₂ML at BTPS conditions was measured by a capnometer built into the Hemolung. Thus, the total carbon dioxide production (VCO₂tot) was calculated as the sum of VCO₂NL and VCO₂ML.

Arterial blood gas analyses (i-STAT; Abbott, USA) and activated clotting time (ACT) (Hemochron Jr. Signature; ITC, USA) were recorded hourly. Moreover, every 3 h, selected electrolytes (sodium, potassium, chloride, and ionized calcium) and glucose concentrations were measured in the arterial samples (i-STAT; Abbott). Mixed venous, extracorporeal circuit blood, and ultrafiltrate samples were collected every 3 h for blood gas analyses. Hematocrit was measured *via* centrifugation technique in mixed venous samples. Heart rate (HR), mean arterial pressure, central venous pressure, and pulmonary artery pressure were monitored continuously. Pulmonary artery occlusion pressure was recorded hourly. Cardiac output (CO) was measured every 3 h using intermittent bolus thermodilution technique.

ML carbon dioxide removal efficiency ratio was computed as described in our previous article (see equations B for ML CO₂ Removal Efficiency Calculation, Supplemental Digital Content 1, <http://links.lww.com/ALN/B237>).¹⁵ ML oxygen delivery (VO₂ML), natural lung oxygen delivery, and total oxygen consumption (VO₂tot) were calculated using standard equations (see equations C for Oxygen Delivery and Consumption Calculation, Supplemental Digital Content 1, <http://links.lww.com/ALN/B237>) at BTPS conditions.

Respiratory quotient (RQ) was calculated as the ratio between VCO₂tot and VO₂tot. Thus, with VCO₂tot and

VO₂tot, EE was calculated using the Weir equation, as previously described (see equations D for Energetic Expenditure Calculation, Supplemental Digital Content 1, <http://links.lww.com/ALN/B237>).¹⁸

Hemoglobin, complete blood count, prothrombin time, partial thromboplastin time, fibrinogen, D-dimers, blood urea nitrogen, creatinine, total bilirubin, alanine transaminase, aspartate transaminase, amylase, myoglobin, uric acid, blood glucose, and plasma-free hemoglobin were measured before instrumentation, at the end of the recovery period (*i.e.*, after 6 h of extracorporeal circulation [EC]), as well as at the end of any ECCO₂R and ALCO₂R phase. Plasma-free hemoglobin concentration was measured by spectrophotometric analysis (DU 800; Beckman Coulter Inc., USA).

Sheep were euthanized by an intravenous injection of 20 ml of Fatal Plus (Vortech Pharmaceuticals, USA) at the conclusion of the experiments. Lung, heart, liver, and renal tissue samples were collected for histological evaluation postmortem. Histological evaluation of injury was performed by a single pathologist blinded to the identity of the animal represented on the slide, as previously documented (see Postmortem Histological Evaluation, Additional Methods, Supplemental Digital Content 1, <http://links.lww.com/ALN/B237>).¹⁹ Moreover, we studied the safety of ALCO₂R technique in terms of tissue inflammatory responses, concentration of selected indices of oxidative stress, and biochemical markers of injury. Specifically, we measured thiobarbituric acid reactive substances (TBARs), interleukin-1 β , interleukin-8, and nitric oxide concentration, as well as determined reduced glutathione and ferric reducing ability in the homogenate of lung, heart, and liver tissue. Myeloperoxidase activity was measured in lung homogenate. Indices of oxidant stress and inflammation were then compared with healthy time controls sham animals from another sheep study performed in our laboratory (see Oxidative Stress and Inflammation Analysis, Additional Methods, Supplemental Digital Content 1, <http://links.lww.com/ALN/B237>).²⁰

Statistical Analysis

Data are expressed as means \pm SD or median and interquartile range, when appropriate. The JMP 11 statistical program (SAS Institute Inc, USA) was used for statistical analysis. A paired difference model was used to gauge the sample size. Using an α error of 0.05 and power of 0.80, with an SD of 5 ml/min in VCO₂ML measurements expected from preliminary experiments, six paired matches were calculated to be needed to detect a difference of at least 7.5 ml/min in VCO₂ML between ALCO₂R *versus* ECCO₂R steps and six animals were sufficient. To further reduce the unnecessary use of animals, we performed two repeated experimental sequences on each of these animals. For systemic variables (*i.e.*, ventilation, hemodynamics, and energetic metabolism) and extracorporeal gas removal (VCO₂ML, VO₂ML, and ML efficiency), a two-way analysis of variance for repeated

measures was performed using a residual maximum likelihood method to fit a general linear model. Treatment (*i.e.*, ALCO₂R *vs.* ECCO₂R, 2 levels) and time (*i.e.*, hours, 12 levels) were considered as fixed factor, whereas animals and sequence repetitions (nested within animals) were considered as random effects. Interactions between treatment and time were not analyzed. For extracorporeal gas analyses, a similar statistical model was used, with treatment (*i.e.*, ALCO₂R *vs.* ECCO₂R, two levels) and circuit withdrawal port (*i.e.*, six levels) as fixed effects. Two-tailed values of *P* less than 0.05 were considered statistically significant. *Post hoc* Student's *t* test with Tukey adjustment was used for multiple comparisons.

Results

During LA infusion (*i.e.*, ALCO₂R phases), VCO₂ML was enhanced by 48% relative to ECCO₂R (55.3 ± 3.1 ml/min *vs.* 37.2 ± 3.2 ml/min; *P* < 0.001). Similarly, ML carbon dioxide removal efficiency was significantly increased (41.3 ± 5.3% *vs.* 24.3 ± 4.2%; *P* < 0.001). No reduction in VCO₂ML (fig. 2) and ML carbon dioxide removal efficiency was observed over time. VO₂ML was similar during ALCO₂R and ECCO₂R (9.8 ± 3.2 ml/min *vs.* 9.5 ± 3.2 ml/min; *P* = 0.17).

We did not observe alterations of ventilatory status during ALCO₂R (see Table 1). MV was similar during ALCO₂R and ECCO₂R, as was respiratory rate and VCO₂NL. TV and AV showed a 7% increase during ALCO₂R. V_d and pulmonary Qs/Qt were not influenced by ALCO₂R. No effect of time was observed on ventilatory variables.

Table 2 shows the metabolic parameters during experimental phases. EE increased by 12% during ALCO₂R.

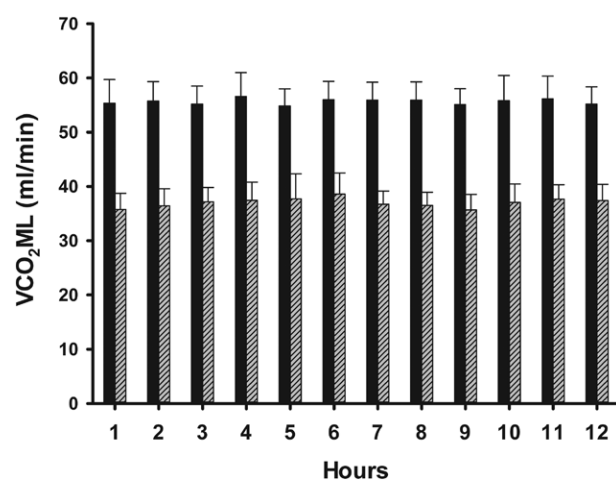


Fig. 2. Extracorporeal carbon dioxide removal (VCO₂ML) during acid load carbon dioxide removal (ALCO₂R) and extracorporeal carbon dioxide removal (ECCO₂R) phases. Black bars represent ALCO₂R phase measurements, whereas hatched bars represent ECCO₂R phase measurements. For any of the time-points, VCO₂ML was higher during ALCO₂R compared with ECCO₂R (*P* < 0.001). Data are expressed as means ± SDs.

Similarly, VCO₂tot, natural lung oxygen delivery, and VO₂tot were 13% higher during ALCO₂R relative to ECCO₂R. RQ was not significantly influenced by ALCO₂R.

Table 3 presents arterial blood gas analyses and electrolyte concentrations measured during experimental phases. During ALCO₂R, pH, Pco₂, bicarbonate ions (HCO₃⁻), and base excess were lower relative to ECCO₂R. Oxygenation was not affected by ALCO₂R. Average lactate level was higher during the ALCO₂R phase compared with ECCO₂R. During the first hour of ALCO₂R, lactate was significantly lower (3.31 ± 0.98 mmol/l; *P* < 0.001) compared with other time-points. After the first hour, lactate plateaued (see fig. S2, Additional Results, Supplemental Digital Content 1, <http://links.lww.com/ALN/B237>). Sodium, potassium, and ionized calcium were not influenced by ALCO₂R, whereas chloride was lower during ALCO₂R. Glucose was higher during ALCO₂R compared with ECCO₂R. No effect of time was observed on arterial blood gas analyses.

The pH, Pco₂, HCO₃⁻, and lactate in the blood and ultrafiltrate samples from the circuit are shown in table 4. During ALCO₂R, the highest lactate was observed in the post-acid ultrafiltrate samples (*P* < 0.001). In postrecirculation, post-ML, and outlet blood samples, lactate was similarly higher relative to inlet blood (*P* < 0.001), which in turn had a lactate concentration similar to the arterial blood. During ALCO₂R, similar to arterial samples, inlet blood samples showed reduced pH, Pco₂, and HCO₃⁻ compared with ECCO₂R. LA infusion significantly acidified post-acid ultrafiltrate and subsequently postrecirculation blood (*P* < 0.001). In turn, these samples showed higher Pco₂ and lower HCO₃⁻ (*P* < 0.001). Acid infusion buffered the extreme iatrogenic alkalosis observed in post-ML and outlet blood during ECCO₂R. Indeed, during ALCO₂R, post-ML and outlet blood samples had similarly lower pH and higher Pco₂ relative to ECCO₂R (*P* < 0.001). Regardless of the experimental phase, post-ML and outlet blood, as well as pre-acid ultrafiltrate, had similar pH, Pco₂, and HCO₃⁻ concentrations. Moreover, in these samples, pH was higher and Pco₂ was lower relative to postrecirculation blood independent of acid infusion (*P* < 0.001). pH, Pco₂, HCO₃⁻, and lactate values remained stable over time.

Hemodynamic parameters are shown in table S1 (Additional Results, Supplemental Digital Content 1, <http://links.lww.com/ALN/B237>). During ALCO₂R, a small increase in core temperature was observed (0.1°C). Moreover, ALCO₂R was associated with a rise of about 5% in both HR and CO. Mean arterial pressure, central venous pressure, pulmonary artery occlusion pressure, mixed venous saturation, and hematocrit were similar during ALCO₂R and ECCO₂R.

Changes observed in blood chemistries were associated with instrumentation and connection to the EC rather than with application of ALCO₂R (see table S2, Additional Results, Supplemental Digital Content 1, <http://links.lww.com/ALN/B237>). No sign of hemolysis was observed because plasma-free hemoglobin concentration was always

Table 1. Ventilatory Parameters during Extracorporeal Carbon Dioxide Removal (ECCO₂R) and Acid Load Carbon Dioxide Removal (ALCO₂R)

	ECCO ₂ R	ALCO ₂ R	P Value
MV (l/min)	7.51 ± 1.89	7.88 ± 2.00	0.146
TV (ml)	292 ± 54	312 ± 51	<0.001
RR (bpm)	26.6 ± 8.9	26.1 ± 9.5	0.568
AV (l/min)	3.37 ± 1.05	3.72 ± 0.87	0.012
VCO ₂ NL (ml/min)	159.6 ± 51.8	167.9 ± 41.6	0.063
V _d (ml)	152 ± 23	156 ± 20	0.122
Qs/Qt (%)	8.8 (7.1–12.6)	9.4 (6.7–13.0)	0.920

AV = alveolar ventilation; MV = minute ventilation; Qs/Qt = venous admixture; RR = respiratory rate; TV = tidal volume; VCO₂NL = natural lung carbon dioxide removal; V_d = physiologic dead space.

Table 2. Metabolic Parameters during Extracorporeal Carbon Dioxide Removal (ECCO₂R) and Acid Load Carbon Dioxide Removal (ALCO₂R)

	ECCO ₂ R	ALCO ₂ R	P Value
VCO ₂ tot (ml/min)	196.6 ± 50.9	223.5 ± 42.7	<0.001
VO ₂ NL (ml/min)	234.1 ± 92.3	256.2 ± 110.0	0.041
VO ₂ tot (ml/min)	228.9 ± 107.8	260.7 ± 117.3	0.036
EE (kcal/h)	67.0 ± 26.8	76.1 ± 30.7	0.005
RQ	0.92 ± 0.33	0.98 ± 0.30	0.170

EE = energy expenditure; RQ = respiratory quotient; VCO₂tot = total carbon dioxide production; VO₂NL = natural lung oxygen delivery; VO₂tot = total oxygen consumption.

Table 3. Arterial Blood Gas Analyses and Electrolytes Concentration during Extracorporeal Carbon Dioxide Removal (ECCO₂R) and Acid Load Carbon Dioxide Removal (ALCO₂R)

	ECCO ₂ R	ALCO ₂ R	P Value
pH	7.449 ± 0.055	7.404 ± 0.051	<0.001
Pco ₂ (mmHg)	35.5 ± 3.6	34.4 ± 3.5	<0.001
HCO ₃ ⁻ (mEq/L)	24.3 ± 3.7	21.2 ± 3.6	<0.001
BE (mEq/L)	0.9 ± 4.4	-2.9 ± 4.4	<0.001
HbO ₂ (%)	97.0 ± 1.2	97.0 ± 1.0	0.545
Po ₂ (mmHg)	100.2 ± 12.7	103.9 ± 9.5	0.002
Lactate (mmol/l)	0.66 ± 0.53	5.2 ± 2.0	<0.001
Sodium (mmol/l)	146.4 ± 3.1	145.6 ± 3.4	0.300
Chloride (mmol/l)	108.5 ± 4.3	106.8 ± 3.3	0.046
Potassium (mmol/l)	4.2 ± 0.5	4.1 ± 0.4	0.469
iCa ⁺⁺ (mmol/l)	1.3 ± 0.1	1.3 ± 0.1	0.234
Glucose (mg/dl)	67 ± 10	77 ± 12	<0.001

BE = base excess; HbO₂ = hemoglobin oxygen saturation; HCO₃⁻ = bicarbonate ions; iCa⁺⁺ = ionized calcium.

lower than pathological thresholds²¹ and bilirubin changes were negligible and lower during ALCO₂R compared with ECCO₂R. We observed a slight elevation in white blood cells and in particular neutrophils after instrumentation. Hematocrit and platelets were lower after instrumentation compared with baseline, as an effect of hemodilution after connection to the extracorporeal circuit. As expected, due to heparin infusion, partial thromboplastin time was elevated after instrumentation and was stable during EC, ALCO₂R, and

ECCO₂R. No alteration in prothrombin time, fibrinogen, or D-dimers was observed during the experiment. Alanine transaminase and aspartate transaminase were not different during ECCO₂R and ALCO₂R, although they were higher than baseline. Creatinine, amylase, uric acid, and myoglobin were in normal ranges during the whole experiment.

During ALCO₂R, the heparin infusion rate was lower (38.2 ± 10.8 IU/kg*h vs. 40.0 ± 13.6 IU/kg*h; *P* < 0.05) and the ACT was higher (279 ± 37 vs. 262 ± 35; *P* < 0.05) compared with ECCO₂R.

Histological examination of lung, heart, liver, and kidney organs did not demonstrate tissue damage (see fig. S3 and table S3, Additional Results, Supplemental Digital Content 1, <http://links.lww.com/ALN/B237>). Biomarkers of inflammation, oxidant stress, and tissue injury are shown in table S4 (Additional Results, Supplemental Digital Content 1, <http://links.lww.com/ALN/B237>). Overall, no clinically meaningful changes were detected in these variables, but treated animals showed a statistically significant reduction in heart TBARs concentration, as well as a statistically significant increase in lung total antioxidant and glutathione, liver interleukin-1β, and lung interleukin-8.

Discussion

We investigated the effects of ALCO₂R with LA on ventilation and metabolism of spontaneously breathing sheep. Feasibility, safety, and efficiency of ALCO₂R technique was confirmed and independently validated in a different species adding to previous experiences in swine. To our knowledge, ALCO₂R has never been attempted before in a conscious, spontaneously breathing animal model. For the first time, the effect of ALCO₂R on energy metabolism was evaluated. Infusion of LA was associated with an increase in EE, such that ALCO₂R use was not associated with a decrease in the animals' MV.

ALCO₂R is an innovative, highly efficient ECCO₂R technique based on extracorporeal blood acidification. It opens up the possibility that ECCO₂R systems could be miniaturized, by allowing similar carbon dioxide removal efficiency at half of the currently used BFs. Previous experiments in swine studied the performance of an ML during ALCO₂R, verifying its effectiveness in raising VCO₂ML by up to 70%.^{11,13,22} The current study was designed to evaluate the ventilatory and metabolic effects of ALCO₂R *via* LA, in the absence of metabolic control, in a different species (*i.e.*, sheep) and in a different laboratory during awake, spontaneously breathing conditions. We confirmed the benefits of extracorporeal blood acidification on ML performance, providing independent validation of the ALCO₂R concept.

Interestingly, we did not detect a significant reduction in MV in this model. This could be explained by the augmentation of caloric intake due to LA infusion. In a previous study involving mechanically ventilated anesthetized swine,²³ LA at 2.5 mEq/min increased VCO₂tot by 5% (*i.e.*, 13 ml/min) relative to an isocaloric glucose infusion, while stabilization

Table 4. Blood Gas Analyses in the Extracorporeal Circuitry during Extracorporeal Carbon Dioxide Removal (ECCO₂R) and Acid Load Carbon Dioxide Removal (ALCO₂R)

		Blood				Ultrafiltrate	
Phase	Variable	Inlet	Postrecirculation	Post-ML	Outlet	Pre-acid	Post-acid
ECCO ₂ R							
	pH	7.410±0.042 ^A	7.448±0.075 ^B	7.832±0.138 ^C	7.849±0.076 ^C	7.838±0.075 ^C	—
	Pco ₂ (mmHg)	42.3±2.4 ^A	35.2±3.1 ^B	15.1±2.4 ^C	14.8±2.0 ^C	15.6±1.5 ^C	—
	HCO ₃ ⁻ (mEq/L)	26.4±3.0 ^A	23.6±3.4 ^B	26.2±4.9 ^A	25.6±4.8 ^A	26.4±4.6 ^A	—
ALCO ₂ R							
	pH	7.370±0.045 ^{*A}	6.986±0.138 ^{*B}	7.489±0.090 ^{*A}	7.485±0.095 ^{*A}	7.492±0.083 ^{*A}	5.296±0.677 ^C
	Pco ₂ (mmHg)	40.5±3.2 ^{*A}	77.8±18.9 ^{*B}	18.3±1.7 ^{*C}	18.3±2.6 ^{*C}	18.1±1.6 ^{*C}	382.1±96.8 ^D
	HCO ₃ ⁻ (mEq/l)	22.9±3.5 ^{*A}	17.7±3.2 ^{*B}	14.0±3.2 ^{*C}	13.7±3.2 ^{*C}	13.8±3.0 ^{*C}	3.4±2.9 ^D
	LAC (mmol/l)	5.2±2.0 ^A	13.1±2.5 ^B	12.3±2.0 ^B	12.2±1.9 ^B	12.7±1.9 ^B	31.7±5.9 ^C

Two-way analysis of variance reporting differences in blood gas analyses due to treatment (*i.e.*, ALCO₂R vs. ECCO₂R) or extracorporeal withdrawal site. ALCO₂R vs. ECCO₂R effect (*i.e.*, means on the same column): **P* < 0.05 vs. ECCO₂R (paired *t* test). Withdrawal site effect (*i.e.*, on the same row): samples from different withdrawal sites of the circuitry (*i.e.*, means on the same row) not sharing the same letter are significantly different (*P* < 0.05) (repeated measurements analysis of variance with *post hoc* Tukey honest significant test).

HCO₃⁻ = bicarbonate ions; LAC = lactate concentration; ML = membrane lung.

of the caloric intake by parenteral nutrition (*i.e.*, glucose infusion) and infusion of insulin was undertaken. The former avoids unwarranted overfeeding, which is known to augment ventilatory needs.²⁴ The latter is useful to overcome the insulin resistance associated with lactate infusion, and thus maintains euglycemia.^{25,26} In previous experiments,¹³ ALCO₂R obtained *via* infusion of LA at 2.5 mEq/min yielded an augmentation in VCO₂ML to 45 ml/min. Taken together, these data suggest that ALCO₂R, despite increasing VCO₂tot slightly, produces a much higher increase in VCO₂ML and thus can potentially decrease patient ventilatory needs if associated with control of metabolism.

In this experiment, we let the animals eat freely and evaluated their ability to autoregulate their energy metabolism. LA infusion at 1.5 mEq/min was associated with a 10 kcal/h surge (*i.e.*, +12%) in EE, matched by an increase in VCO₂tot of 26.9 and in VO₂tot of 31.8 ml/min (*i.e.*, +13%). HR and CO rose 5% to support this increment in oxygen consumption. Such augmentation in carbon dioxide production increased ventilatory needs (*i.e.*, 7% increases in TV and AV). The sheep significantly compensated for the caloric input associated with LA, albeit not thoroughly. Indeed, because LA at 1.5 mEq/min corresponds to a caloric intake of 30 kcal/h, LA oxidation could have caused a much higher surge in EE (*i.e.*, +44%). This, added to the 67 kcal/h measured during the ECCO₂R phases, may have led to an eventual EE of about 100 kcal/h during LA infusion. Thus, by the Weir equation, we can extrapolate a theoretical VCO₂tot and a VO₂tot during ALCO₂R phases of 320 ml/min each. Such augmentation in VCO₂tot would have doubled AV, whereas it only increased by 7%. Thus, sheep spontaneously managed the LA caloric input to the point of avoiding metabolic and ventilatory derangements, although without intervention to control their metabolism we were not able to observe the effects of ALCO₂R on ventilation. Notably, the caloric effects of LA infusion may differ in patients on controlled diet whose caloric metabolism and endocrine milieu

may be deranged by underlying critical illness.²⁷ Thus, further studies targeting the metabolic and endocrine responses to ALCO₂R are needed before translation of these results into clinical practice may be done.

The use of special techniques (*e.g.*, isotopic carbon-labeled glucose or lactate, direct calorimetry, and calculation of caloric intake from dietary consumption) would be necessary to investigate the metabolic fate of lactate and glucose during ALCO₂R. This goes beyond the scope of this study. We did observe that RQ shifted from 0.92±0.33 to 0.98±0.30 from ECCO₂R to ALCO₂R, although not significantly. Insofar as the RQ of LA is 1, we may argue that this shift reflected oxidation of LA into carbon dioxide. Moreover, higher glucose levels were observed during LA infusion. This might be explained by the insulin resistance associated with infusion of LA.²⁵

During ALCO₂R, we did not observe blood chemistry alterations, hemodynamic derangements, or hemolysis. Notably, this study is the first report of in-depth analysis of effects of ALCO₂R on lung function, histopathology, and tissue inflammation. ALCO₂R did not have any detrimental effects on V_d, Qs/Qt, and oxygenation. During LA infusion, we detected a slight increase in core temperature. A thermogenic effect is known to be associated with LA metabolism.²⁸ Interestingly, despite achieving target ACT levels, we noticed a reduction in anticoagulation requirements during ALCO₂R. Postmortem histological examination of the heart, liver, lung, and kidneys did not demonstrate major signs of tissue damage. Interestingly, a previous work²⁹ by our group showed similar lung histology in control animals subjected to multiday experiments. The indices of oxidative and nitrosative stress, as well as proinflammatory cytokines, suggest that ALCO₂R does not invoke an inflammatory response in the lung or in other organs such as the heart or liver. This was also supported by the observation of no significant elevation in lung myeloperoxidase activity, lung total antioxidant status, or heart TBARS. Taken together

with the plasma biomarkers of tissue function and lack of histological evidence of tissue injury, these data suggest that ALCO₂R is safe. Nevertheless, we cannot exclude the possibility that ALCO₂R may have detrimental effects. The exposure of blood to reduced pH—even for a brief period—may have lasting consequences.³⁰ Indeed, acidosis is known to inhibit chemotaxis and bactericidal functions of polymorphonuclear leukocytes, as well as cytotoxicity and proliferation of lymphocytes. Similarly, platelet aggregation may be impaired by exposure to lowered pH.^{31,32} In contrast, complement protein activation and antibody binding to leukocytes are enhanced during acidosis.³³ Thus, further studies including control animals are needed to determine whether these aspects of the safety profile would be preserved under conditions as a treatment for lung injury or in subjects experiencing infectious diseases.

Direct infusion of acids would have had deleterious effects on blood. To limit such consequences, we utilized an innovative approach to achieve extracorporeal acidification.¹⁴ Briefly, a hemodiafilter is interposed in the extracorporeal circuit after the ML and ultrafiltrate was generated with a peristaltic pump. This ultrafiltrate is acidified and then recirculated before the ML, allowing highly concentrated hyperosmolar acids to be injected into the recirculating ultrafiltrate. Direct injection of concentrated acids into the blood would have caused hemolysis, while on the contrary, a high volume of free water would have been necessary to infuse isotonic acids, causing severe electrolyte derangements. Both these complications are limited by our approach. Notably, we detected plasma-free hemoglobin levels even lower than the ones measured during our previous experiment,⁵ where the sole Hemolung device was used.

The impact of LA infusion on acid–base homeostasis and electrolyte equilibrium was minor. In effect, we observed a steady level of plasma lactate after the first hour of ALCO₂R, thus suggesting that LA was readily cleared from systemic circulation. Despite the fact that we provided LA at a lower dosage by weight compared with previous swine experiments (0.045 *vs.* 0.057 mEq · min⁻¹ · kg⁻¹), we nevertheless observed higher lactate plasma levels. This can be ascribed to the lower lactate clearance of sheep (*i.e.*, about 10 ml · min⁻¹ · kg⁻¹)³⁴ in contrast to swine (*i.e.*, about 20 ml · min⁻¹ · kg⁻¹)²³ and to the small size of our experimental animals. Although human lactate clearance by weight is similar to that of sheep (9 to 12 ml · min⁻¹ · kg⁻¹),^{35–37} in an adult man (*i.e.*, weight 70 kg), LA infusion at 1.5 mEq/min (*i.e.*, 0.021 mEq · min⁻¹ · kg⁻¹) would lead to an acceptable rise in arterial lactate up to 1.7 mEq/L.

We consider this experiment to be a “stress test” of the ALCO₂R technique. In this study, animals with intrinsically limited clearance capacity for lactate and small weight underwent a high-dose ALCO₂R technique without any corrective intervention to stabilize their metabolism. Despite this, in this challenging scenario, ALCO₂R was safe with respect to organ function, electrolyte equilibrium, and

acid–base homeostasis, and at least as effective as the standard ECCO₂R technique. We suggest that ALCO₂R should be coupled with metabolic control obtained by euglycemia, as well as with the reduction of caloric intake. This hypothesis will need to be tested in large animal models of lung injury. Moreover, future studies are warranted to evaluate the efficacy of ALCO₂R on a clinically relevant model of hypercapnia and the safety profile of the technique with regard to impairment of immunologic function, platelet aggregation, and activation of complement.

Several solutions may be used to further ameliorate ALCO₂R technique. Other metabolizable acids may be used (*e.g.*, citric and acetic). These compounds may provide advantages other than acidification, such as blood anticoagulation¹⁴ or a more favorable metabolic profile.³⁸ During ALCO₂R, partial or total clearance of the infused acid may be desired. If so, the extracorporeal circuit we used may be easily supplemented with additional devices for ultrafiltrate removal and/or fluid replacement. Such a circuit would not only promote carbon dioxide removal but also provide blood purification and volume control, as well as clearance of the infused acid. Thus, in the setting of multiorgan failure (*e.g.*, renal and pulmonary), *modular* multiorgan support technology¹⁵ can be envisioned. Last, nonmetabolizable acids may be used (*e.g.*, hydrochloric acid), thus avoiding the caloric effects due to infusion of metabolizable acids. However, infusion of a nonmetabolizable acid would result in progressive accumulation of the parent anion (*e.g.*, Cl⁻),³⁹ thus requiring the development of further extracorporeal techniques to remove it. In this regard, we recently elaborated an ECCO₂R technique based on electrodialysis of plasmatic water called respiratory electrodialysis capable of selectively modulating chloride concentration in the extracorporeal circuit.⁴⁰ In a mechanically ventilated swine model, respiratory electrodialysis proved efficient in enhancing VCO₂ML and controlling ventilation.

In conclusion, feasibility and effectiveness of extracorporeal blood acidification in enhancing carbon dioxide removal by a ML was confirmed in a different species and by an independent laboratory. Moreover, the ALCO₂R technique was demonstrated to be safe. However, infusion of LA without metabolic control caused a rise in EE that made ALCO₂R no different from standard ECCO₂R with respect to ventilation. We suggest that LA-enhanced ALCO₂R should be coupled with active measures to control metabolism. This technology is being further developed to permit its application in humans.

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Competing Interests

We acknowledge the following potential conflicts of interest. Dr. Pesenti received payment for lectures and service on speaker bureau from Maquet (Maquet Cardiopulmonary, Rastatt, Germany) and Novalung (Novalung, Heilbronn, Germany), received consulting honoraria from Maquet (Maquet Cardiopulmonary, Rastatt, Germany) and Novalung (Novalung, Heilbronn, Germany), and has patents pending or issued (WO2008EP03661, co-owned with Università Milano-Bicocca [Milano, Italy]; IT2012BO00405; IT2012BO00404). Dr. Batchinsky received support for travel and consulting honorarium from ALung Technologies (ALung, ALung Technologies, Pittsburgh, Pennsylvania) and Maquet (Maquet Cardiopulmonary, Rastatt, Germany). Dr. Cancio disclosed that this research was supported, in part, by his appointment to the Knowledge Preservation Program at the U.S. Army Institute of Surgical Research, administered by the Oak Ridge Institute for Science and Education, through an interagency agreement between the U.S. Department of Energy and the U.S. Army Medical Research and Materiel Command. The remaining authors stated that they do not have any potential conflicts of interest. The opinions or assertions contained herein are the private views of the authors and are not to be construed as official or as reflecting the views of the Department of the Army or the Department of Defense.

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